subtype of $GABA_A$ receptors. Optogenetic control of protein functions has been an emerging approach for studying cell signaling. Here we present a novel approach for modulating $GABA_A$-receptor activities. The approach involves two players: (1) an engineered $GABA_A$ receptor with a cysteine properly positioned near the GABA binding site; and (2) a photoswitch that can be covalently attached to the designated cysteine. The photoswitch consists of a cysteine-reactive maleimide, a photosensitive azobenzene, and an antagonist of the receptor. Receptor inhibition is turned on and off upon light switching.
Several candidate mutants and photoswitches were identified from an extensive screening on recombinant $GABA_A$ receptors ($\alpha1\beta2$ and $\alpha5\beta2$) using electrophysiology. The "optogenetic receptors" (i.e., the photoswitch-labeled mutants) exert rapid, reversible changes in the amplitude of GABA-evoked currents in response to light switching. When the attached photoswitch is converted to the *trans* state by 500-nm light, the receptor becomes less sensitive to GABA (manifested in an increased EC_{50}) compared to the unlabeled receptor. The activity of the inhibited receptor is restored upon 380-nm illumination. These optogenetic $GABA_A$ receptors are potentially useful tools for studying synaptic and extrasynaptic GABAergic transmission in the mammalian brain.

129-Plat
Ion Access Pathway to the Transmembrane Pore in P2X Receptors
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P2X receptors are ATP-gated channels that mediate cation influx to evoke action potentials or trigger intracellular signaling, important in sensory signal transmission and inflammatory responses. However, the molecular mechanism of how cations penetrate the extracellular region of the receptor and access the transmembrane pore is unknown. Examination of the closed state crystal structure predicts two potential pathways: i) three fenestrations located above the membrane (lateral pathways) and ii) the central void along the molecular three-fold axis of symmetry (central pathway). By solving the Poisson-Boltzmann equation, we analyzed the electrostatics of cations along these pathways in the closed state, and found that relatively small widening (~1 Å) seemed sufficient to allow cations to penetrate the lateral pathways, while even a large displacement (> 5 Å) along the central pathway remained unfavorable for cations to go through. To probe the accessibility in the open state, we systematically introduced cysteine residues into the rat P2X2 receptor along these potential access pathways and measured reaction rates with Cys-reactive reagents using patch-clamp recording techniques. Among 47 mutants examined, only I328C along the lateral pathway was modified by MTSET and MTS-TPAE, suggesting that cations do not utilize the central pathway. To further investigate the motion during activation, we cross-linked neighboring subunits by introducing disulfide-bridges at 5 different positions along the central pathway. While the disulfide-bridges at the three middle positions did not affect currents, the top pair exhibited reduction and the bottom pair showed potentiation when treated with reducing agents. These indicate that the central pathway seems unlikely to become wider, except the bottom part that is directly connected to the lateral pathways. Altogether, these results suggest that the lateral pathways seem to "breathe" during activation, allowing extracellular cations to penetrate and access the transmembrane pore.

130-Plat
Opening the Pore of ASIC1a
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Acid-sensing ion channels (ASICs) are voltage-independent proton-gated ion channels expressed throughout neurons of the mammalian central and peripheral nervous systems. These channels are organized as homo- or hetero-trimers. Each subunit has two transmembrane segments (TMs) connected by a large extracellular region with the N- and C- termini on the intracellular side. The underlying mechanism coupling proton binding in the extracellular region to pore opening is unknown. To define structural rearrangements associated with gating and desensitization in the outer vestibule of the pore of ASIC1a, we explored the reactivity toward methanethiosulfonate (MTS) reagents of channels bearing Cys substitution in individual residues of the tract I426-Q436. Mutant channels were expressed in Xenopus oocytes and the activity was studied with the two-electrode voltage clamp technique. We found that positively-charged MTS reagents trigger pore opening of G428C in the absence of extracellular acidification. Scanning mutagenesis and double-mutant cycle analysis indicated that the covalently modified side chain of Cys at position 428 interacts with Tyr424 in the region preceding TM2. A Tyr424 to Phe substitution disrupted proton-dependent gating, although pore opening was triggered by MTSET. Our results suggest that the region preceding TM2 provides functional coupling between the extracellular proton-binding sites and the ion pore. Strikingly, Y424C-G428C monomers were associated by inter-subunit disulfide bonds and were insensitive to MTSET. Conformational restrictions on the outer vestibule of the pore arising from these inter-subunit 424-428 disulfide bonds do not prevent the channel from cycling through the resting, open and desensitized states. Our studies suggest that the opening of the ion conductive pathway involves coordinated rotation of the TM2 helices.

131-Plat
D433 Does Not Determine Ion Selectivity in ASIC1
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ASICs (Acid Sensing Ion Channels) are ion channels activated by external protons. They belong to the family of ion channels known as ENaC/Degenerin, which has a distinctive high selectivity for Na+ ions. The atomic structure of chicken ASIC1 solved by Gonzales (Gonzales et al., Nature 460: 599604, 2009) shows the transmembrane segments, TM1 and TM2, of three subunits around the pore. The TM2s are tilted ~50 degrees to the normal of the membrane producing a constriction that closes the lumen of the pore near the external side of the ion pathway. The side chain of D433 in TM2 defines the narrowest segment of the pore in the desensitized state and the carboxyl group coordinates a Cs+ ion, suggesting that D433 forms both the desensitization gate and the selectivity filter. We show here that most substitutions of residue D433 reduce or abolish ASIC1 currents and change the kinetics of desensitization. The polar residues Q and N decrease the mean duration of open events to a few milliseconds but maintain the ion selectivity sequence: Na+>Li+>K+>>>Cs+, and the value of the unitary conductance: 17 pS (120 mM symmetric Na+, V range −80 to −20 mV) indicating that D433 participates in shutting the pore but not in setting ion selectivity. Hence, the narrowest segment of the ion pathway is not the same in the open and desensitized states. Together, the results predict a significant change in the architecture of the transmembrane segments when the channel adopts the open conformation.

SYMPOSIUM 5: Phospholipid Modulation of Ion Channels

132-Symp
Voltage-Sensitive PIP2 Phosphatase Shows PIP2 Requirements for KCNQ and Ca^{2+} Channels
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The four talks in this symposium highlight the dependence of ion channel function on membrane phospholipids and fatty acids. Although of low abundance (1-2%), the phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP_2) is a signature lipid of plasma membranes. The PIP_2 lipid aids ion channel function there, and depleting PIP_2 can decrease the activity of >80 types of channels (Logothetis et al, Pflügers 2010). A voltage sensitive 5-phosphatase (VSP) converts PIP_2 to PI(4)P rapidly during strong depolarizing voltage steps (Okamura et al, J Physiol 2009). Exogenously expressed, this VSP serves to test the PIP_2 dependence of plasma membrane ion channels and the kinetics of resynthesis of PIP_2. Using the PLCdelta1 PH (pleckstrin homology) domain as a reporter, we measure the membrane PIP_2 concentration as a change in FRET (Förster resonance energy transfer) between YFP-PH and CFP-PH. The PH-domain assay reports that strongly activated VSP depletes PIP_2 with a time constant <150 ms. Activating VSP for 500 ms with a +100 mV depolarization reduces current in KCNQ channels by 90% (Falkenburger et al, JGP 2010) and reduces current in four subtypes of voltage-gated Ca^{2+} channels by 30-60% (Suh et al, Neuron 2010). The channel currents and the PH domain signals recover with time constants of 7-15 s. Such observations imply that: (1) KCNQ channels and several Ca^{2+} channels need PIP_2 for best operation, (2) the PIP_2 likely binds directly to the channel protein, (3) PH domains and channels form low-affinity complexes with PIP_2 that last < 10 ms, and (4) resynthesis of PIP_2 from plasma membrane PI(4)P takes only ~10 s. This resynthesis from PI(4)P is much faster than the 150-s time seen for two-step resynthesis from PI after activation of receptors coupled to PLC. Support: NIH grants NS08174, GM83913.